PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alison Joy HODGKINSON et al. Attorney Docket: P64057US2

Serial No. 10/067,870 Group Art Unit: 1644

Filed: February 8, 2002 Examiner: Michael Edward SZPERKA

For: PROCESS FOR THE PRODUCTION OF IMMUNOGLOBULIN A IN MILK

DECLARATION UNDER 37 C.F.R. § 1.132

1. I, Colin Prosser, am a citizen of Australia and reside at 33 Taplin Road, RD3, Hamilton, New Zealand.

2. I am a senior scientist of AgResearch, Ruakura Agricultural Center, Hamilton, New Zealand. I specialize in lactational physiology with special emphasis on composition of milk from different species. During the last 25 years, the scientific research I conducted or participated includes the research in the fields of compositional changes in breast milk during menstrual cycle and pregnancy, IGF-I and its binding proteins in human, bovine and caprine milk, and molecular and physiological mechanisms underlying the variations in milk composition in diary animals, etc.

3. I am familiar with the U.S. patent application Serial No. 10/067,870 ('870 application).

4. The experiments conducted according to the '870 application include the steps of:
(a) actively immunizing a pregnant ruminant mammal with an antigen by any two routes of administration selected from the group consisting of intramammary (IMM), intraperitoneal (IP), and intramuscular (IM); and
(b) actively immunizing said ruminant mammal with an antigen by a third administration route selected from the group consisting of intramammary (IMM), intraperitoneal (IP), and intramuscular (IM); wherein all three administration routes are different.

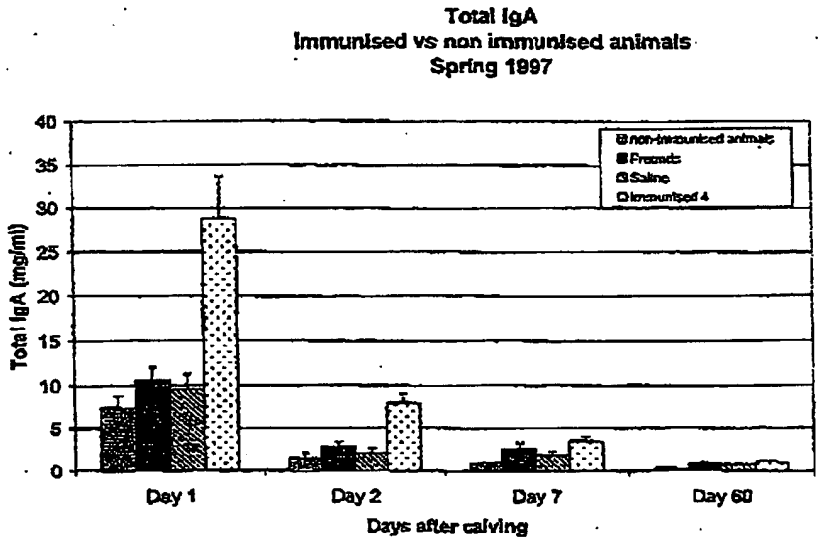
5. It is respectfully submitted that experiments conducted according to the invention described in the '870 application show that the total IgA in milk reaches an elevated level that is not able to be achieved by only actively immunizing a pregnant ruminant mammal with an antigen by any two routes of administration selected from the group consisting of intramammary (IMM), intraperitoneal (IP), and intramuscular (IM).

6. In all the experiments conducted according to the '792 application, the total IgA in milk or colostrum was measured by ELISA using antibody to bind bovine IgA specifically. The ELISA measures all IgA, irrespective of the antigen specificity of the IgA antibody.

7. In an experiment conducted in 1997, Friesian or Jersey cows that had completed at least one lactation cycle were used. Antigens used in the experiment include Cocktail antigent–Escherichia coli K88, Candida albicans, Helicobacter pylori, Escherichia coli O157, Clostridium difficile; and single antigen – Candida albicans. In the experiment, 10 cows were immunized by the intraperitoneal (IP), intramuscular (IM) and intramammary (IMM) routes. However, one udder half was immunized with saline + antigen and other udder half with Freund's Incomplete Adjuvant + antigen. IgA concentrations were compared with 27 cows immunized using the regular intraperitoneal (IP), intramuscular (IM) and intramammary (IMM) routes, but with a cocktail of 5 antigens. 5 cows were not immunized and used as controls. The immunizations were given according to the following schedule, where 0 weeks was the expected time of calving.

-8 weeks	-4 weeks	-2 weeks	-1 week
IP/IM	IP/IM/IMM	IMM	IP/IM

8. The experiment results are shown in the following chart and table:



Total IgA	non-immunized animals		Freunds		Saline		immunized cocktail	
	Mean	sem	Mean	sem	Mean	sem	Mean	SEM
Day 1	7.435	1.337265	10.53	1.61	9.62	1.68	28.77	4.87
Day 2	1.4125	0.589052	2.69	0.65	2.05	0.50	7.95	1.04

Day 7	0.89	0.076667	2.60	0.62	1.78	0.45	3.48	0.50
Day 60	0.32625	0.090591	0.94	0.12	0.77	0.11	1.00	0.15

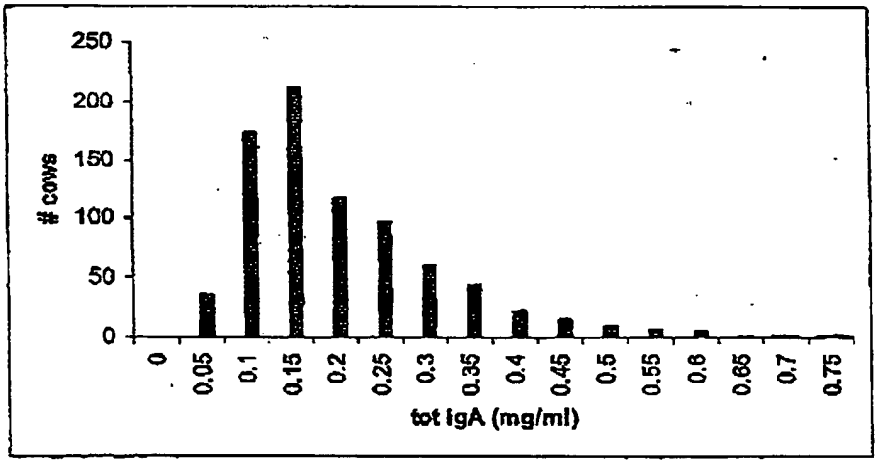
IgA concentrations were nearly 3 fold higher at day 60 following calving in the milk from udder halves infused with Antigen + Fruends incomplete adjuvant, compared with milk from non-immunized animals.

9. In an experiment conducted in 2001, the antigen used for this experiment was *Candida albicans* strain ATCC 10231. Immunogen was made up from the antigen suspension in sterile 0.9% saline and Freunds Incomplete Adjuvant. Approximately 900 multiparous animals (mix of Friesian or Jersey) were treated. Animals were immunized by the intraperitoneal (IP), intramuscular (IM) and intramammary (IMM) routes. The first immunisation was given to all cows approximately 8 weeks before the expected calving date of the earliest calving cow in the herd. Other immunisations followed as per the schedule below:

-8 weeks	-4 weeks	-2 weeks	-1 week
IP/IM	IP/IM/IMM	IMM	IP/IM

Milks from individual animals were obtained at the first scheduled herd test for each farm. The length of time animals had been milking on the herd test day ranged from 12-85 days, depending on their calving date.

10. The experiment results are shown in the following chart and table: Histrograph showing distribution of total IgA concentrations amongst all cows.



The average concentration of IgA for all the cows was 0.22 g/l, ranging from less than 0.1g/l to 0.75 g/l. 21% of cows had values of IgA less than 0.1 g/l IgA. The concentration of IgA in non-immunized cows in this season were 0.08 mg/ml. Note: IgA assay and standard used to measure total IgA was different compared with 1997 trial.

11. In another experiment conducted in 2001, 8 cows immunised by the standard regime, using Candida albicans mixed with Fruends incomplete adjuvant as the immunogen.

12. The experiment results are shown in the following table:

Day after calving (d)	Milk Yield (kg/d)	IgA (mg/ml)	IgA yield (g/d)
1	6.9	4.42	24.0
2	7.1	0.94	6.7
7	10.5	0.25	2.5
14	12.6	0.30	3.5
28	12.9	0.25	2.9
60	13.0	0.21	2.6
119	12.2	0.29	3.4
123	11.3	0.31	3.3
131	12.3	0.31	3.5
138	11.6	0.34	3.9
140	11.4	0.35	4.0

IgA concentrations were maintained through to day 140 following calving

Output at day 140 approx. 15% higher than day 60.

13. Therefore, the above experiments conducted according to the invention described in the '870 application show that the total IgA in milk reaches an elevated level [that is not able to be achieved by only actively immunizing a pregnant ruminant mammal with an antigen by any two routes of administration selected from the group consisting of intramammary (IMM), intraperitoneal (IP), and intramuscular (IM)].

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

EXECUTED at HAMILTON this 21st day of APRIL, 2005.

By C G Prosser
Colin Prosser, Ph.D.

CURRICULUM VITAE

Full name:
Dr Colin Prosser

Nationality
Australian

Present position:
Senior Scientist, AgResearch, Hamilton, New Zealand.

Professional speciality:
Lactational physiology with special emphasis on composition of milk from different species.

Present research interest
Current research is focused on characterising the functional benefits of milk based products, developing technologies to enhance bioactive ingredients in milk and validation of the benefits these provide to the consumer.

Academic qualifications:

BSc (Hons 1), Biochemistry	University of Western Australia	1980
PhD, Human lactation	University of Western Australia	1984

Professional positions held:
- Visiting Research Scientist, National Institutes of Health, USA, 1983-1986
- Postdoctoral Research Scientist, AFRC Institute of Animal Physiology and Genetics Research, UK 1986-1987
- Postdoctoral Research Scientist, University of Nottingham, UK, 1987-1990
- Research Scientist, Dairying Research Corporation), NZ 1990-1994
- Research Scientist, AgResearch, NZ 1995-present

Teaching
- Tutor in general biochemical topics to 2nd and 3rd year Medical, Dental and Science students, University of Western Australia
- Supervision of PhD and MSc students at University of Nottingham, Waikato, Massey and Otago Universities
- Honorary Lecturer in the Department of Biochemistry, University of Otago
- Visiting Lecturer (Lactational Physiology), Waikato University

Research activity.
1980-1983
Department of Biochemistry, University of Western Australia
PhD on compositional changes in breast milk during menstrual cycle and pregnancy. Significant alterations in milk and saliva composition were found to be associated with ovulation and pregnancy.
1983-1986
National Institutes of Health, USA
The regulation of glucose transport system of the murine mammary epithelial cell was characterised using *in vitro* explant culture. The minimal hormonal requirement was insulin (or insulin-like growth factor-I), glucocorticoid and prolactin.

1986-1987

AFRC Institute of Animal Physiology and Genetics Research, Cambridge, UK

The nature and origin of IGF-I and its binding proteins in human, bovine and caprine milk were investigated. Concentrations of IGF-I milk varied greatly between species and with stage of lactation.

1987-1990

Department of Physiology and Environmental Science, University of Nottingham, UK – This placement was based at Babraham, Cambridge

The physiological action of growth hormone in the lactating ruminant was investigated. Results demonstrated the essential role for increased supply of IGF-I to the mammary gland for the galactopoietic response to growth hormone treatment. The role of binding proteins in IGF function was also investigated in goats.

1990-present

AgResearch, Ruakura Agricultural Centre, Hamilton, NZ

Research to define the action of IGF-I and related peptides *in vivo* were continued in lactating goats. The role of microcirculation in the mammary gland in determining milk yield was also investigated.

From 1994, research was re-directed to the molecular and physiological mechanisms underlying the variations in milk protein composition in dairy animals. Technology allowing rapid and high throughput assay of individual proteins in milk for the dairy industry was developed and commercialised.

This knowledge is now directed to identification and improvement of the functional properties of milk. This includes the manipulation of regulatory pathways to enhance the concentration of key functional ingredients in milk and validation of the benefits these provide to the consumer.

The two research programmes focus on defining the unique nutritional and bioactive components of goat milk for development of innovative nutritional and functional formulations and to understand IgA production in the mammary gland to support development of a commercial venture enhancing IgA antibodies against specific gut pathogens in milk.

Publications

Co-editor on two books related to Biotechology in dairying

Published 65 research articles

Invited presentations to 15 international conferences

Numerous industry reports currently used in industry marketing brochures

Selected publications

PROSSER, C MCLAREN, R RUTHERFURD, S DARRAGH, A HENDRIKS, W & LOWRY, D (2003)
Digestion of milk proteins from cow or goat milk infant formula. *NZ Society of Paediatrics, Queenstown, NZ.*

PROSSER C, STELWAGEN K, CUMMINS R, GUERIN P, GILL N & MILNE C (2003)
Reduction in heat induced gastrointestinal hyperpermeability by bovine colostrum and goat milk powders. *Journal of Applied Physiology* (Article in Press – DOI, 10.1152/japplphysiol.00295.2003).

COLLIN R G, PROSSER C G, McLAREN R, THOMSON M & MALCOLM D B (2002) Development and validation of a nephelometric immunoassay for IgG1 in milk. *Journal of Dairy Research* **69**: 27-35.

FARR, V.C., PROSSER, C.G., CLARK, D.A., TONG, M. COOPER, C.V., WILLIX-PAYNE,D., DAVIS, S.R. (2002)
Lactoferrin concentrations are increased in milk from cows milked once-daily. *Proceedings of the New Zealand Society of Animal Production*. 62: 22-23.

WILLIX-PAYNE DJ, PROSSER CG, HODGKINSON AJ, HUTCHINSON J, CANNON RD, HOLMES AR & FISCHER FJ (2001).
Novel Immunoglobulins. Global Bioactive Summit, Hamilton, July 2001

PROSSER C, HURFORD D, MCLAREN R, WILLIX-PAYNE D & LOWRY D (2001)
New Zealand Goat Milk Reduces Gut Damage by Indomethacin. Poster paper, IDF Conference, Auckland, October 2001.

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Gut Damage by Indomethacin is reduced by New Zealand Goat Milk. Global Bioactive Summit, Hamilton, July, 2001.

WELCH, RAS BURNS, DJW DAVIS, SR POPAY A I & PROSSER, CG
Milk Composition, Production and Biotechnology.
Butterworths; London (1997)

PROSSER C G (1996)
Insulin-like growth factors in milk and mammary gland. *Journal of Mammary Gland Biology and Neoplasia* **1**:297-306.

PROSSER C G, DAVIS S R, FARR V C & LACASSE P (1996)
Regulation of blood flow in the mammary microvasculature. *Journal of Dairy Science* **79**:1184-1197.

WHEELER T T, CALLAGHAN M R, PROSSER C G, DAVIS S R & WILKINS R J (1995)
Milk protein synthesis, gene expression and hormonal responsiveness in primary cultures of mammary cells from lactating sheep. *Experimental Cell Research* **217**:346-354.

PROSSER C G (1988)
Mechanism of the decrease in hexose transport by mouse mammary epithelial cells caused by fasting. *Biochemical Journal* **249**:149-154.

GILMOUR R S, PROSSER C G, FLEET I R, COCCO L, SAUNDERS J C, BROWN K D & CORPS A N (1988)
From animal to molecule: aspects of the biology of insulin-like growth factors. *British Journal of Cancer* **58(suppl 9)**:23-30.

HARTMANN P E & PROSSER C G (1984)
Physiological basis of longitudinal changes in human milk yield and composition. *Federation Proceedings* **43**:2448-2453.

HARTMANN P E, RATTIGAN S, PROSSER C G, SAINT L & ARTHUR P G (1984)
Human lactation: back to nature. In: Physiological Strategies of Lactation. Symposia of the Zoological Society of London 51:337-368.